FORMULATIONS FOR BOVINE GRANULOCYTE COLONY STIMULATING FACTOR AND VARIANTS THEREOF

Granulocyte Colony Stimulating Factor (G-CSF) is a member of the growth hormone supergene family. G-CSF stimulates the proliferation of specific bone marrow precursor cells and their differentiation into granulocytes. Furthermore, G-CSF is a potent stimulus for neutrophil proliferation and maturation in vivo (Cohen et al., Proc. Natl. Acad. Sci. 1987; 84: 2484-2488; see also Heidari et al., Vet. Immol Imunopathol. 2001; 81:45-57). G-CSF is also capable of inducing functional activation or "priming" of mature neutrophils in vitro (Weisbart, R. H. et al., Annals of Internal Medicine 1989; 110:297-303). G-CSF has been shown to prime human granulocytes and enhance superoxide release stimulated by the chemotactic peptide N-formyl-methionyl-leucyl-phenalalanine (S. Kitagawa, et al., Biochem. Biophys. Res. Commun. 1987; 144:1143-1146, and C. F. Nathan, Blood 1989; 74:301-306), and to activate human neutrophil IgA mediated phagocytosis (Weisbart, R. H., et al., Nature 1988; 332: 647-649).

G-CSF has been found to be useful in the treatment of indications where an increase in neutrophils will provide benefits. G-CSF is also useful alone, or in combination with other compounds (such as other cytokines) for growth or expansion of cells in culture, for example, for bone marrow transplants.

The cDNA cloning and expression of recombinant human G-CSF (hG-CSF) has been described, and the recombinant hG-CSF exhibits most, if not all, of the biological properties of the native molecule (Souza, L. et al., Science 232, 61-65 (1986)). Sequence analysis of the cDNA and genomic DNA clones has allowed the deduction of the amino acid sequence and reveals that the protein is 204 amino acids long with a signal sequence of 30 amino acids. The mature protein is 174 amino acids long and possesses no potential N-linked glycosylation sites but several possible sites for O-linked glycosylation.

Pharmaceutical preparations containing hG-CSF are known in the art and include numerous formulations. For example, various formulations of hG-CSF are described in Piedmonte et al., Advanced Drug Delivery Reviews, 60: 50-58 (2008), Herman et al., in Formulation, Characterization, and Stability of Protein Drugs, Rodney Pearlman and Y. John Wang, eds., Plenum Press, New York (1996), U.S. Pat. No. 5,919,757 to Michaelis et al., and U.S. Pat. No. 6,908,610 to Sato et al. Traditionally, surfactants are included in hG-CSF formulations and may protect hG-CSF at potentially destabilizing interfaces, against surfaces encountered during processing, and against the alteration of its conformational stability.

The cDNA cloning and expression of recombinant bovine G-CSF (bG-CSF) has also been described. For example, the polynucleotide and polypeptide sequence of mature bG-CSF is presented in U.S. Pat. No. 5,849,883, which also describes methods to clone, isolate, and purify the polypeptide and analogs thereof. Mature bG-CSF is 174 amino acids in length and has 82% homology to hG-CSF. Heidari et al., supra, describe the expression, purification, and biological activities of bG-CSF.

Administration of bG-CSF to cattle can provide therapeutic benefits. Accordingly, a pharmaceutical formulation containing bG-CSF is desirable to utilize its therapeutic potential. However, bG-CSF pharmaceutical formulations developed according to traditional methods known in the art result in undesirable product properties, such as aggregation and destabilization of the bG-CSF polypeptide and/or the formulation.

Therefore, there exists a need for a stable bG-CSF pharmaceutical formulation with desirable properties, such as minimal product aggregation and destabilization properties. Accordingly, the present invention provides stable aqueous pharmaceutical formulations with a bG-CSF polypeptide or a variant thereof which exhibit desirable properties and provide related advantages as well.

This invention provides stable aqueous formulations comprising a bG-CSF polypeptide or a variant thereof, a buffer substance, and an excipient, wherein said formulation is substantially free of polyoxyethylene (20) sorbitan monolaurate. The invention also provides methods of using, a lyophilized or powdered form of, and processes for preparing the formulation.

The stable aqueous formulations of bovine granulocyte colony stimulating factor ("bG-CSF") according to the invention contain a bG-CSF polypeptide or a variant thereof. As used herein, "bovine G-CSF polypeptide" (alternatively referred to as "bG-CSF polypeptide," "bovine G-CSF," or "bG-CSF") and variants thereof shall include those polypeptides and proteins that have at least one biological activity of a CSF, bG-CSF analogs, bG-CSF mutants, altered glycosylated bG-CSF, PEG conjugated bG-CSF, bG-CSF isoforms, bG-CSF mimetics, bG-CSF fragments, hybrid bG-CSF proteins, fusion proteins, oligomers and multimers, homologues, glycosylation pattern variants, variants, splice variants, and muteins, thereof, regardless of the biological activity of same, and further regardless of the method of synthesis or manufacture thereof including, but not limited to, recombinant (whether produced from cDNA, genomic DNA, synthetic DNA or other form of nucleic acid), in vitro, in vivo, by microinjection of nucleic acid molecules, synthetic, transgenic, and gene activated methods. Additionally, the term bG-CSF polypeptide or a variant thereof encompasses bG-CSF polypeptides comprising one or more amino acid substitutions, additions or deletions. See U.S. Pat. No. 5,849,883 for examples of analogs of bovine G-CSF. The sequence of mature bG-CSF polypeptide is 174 amino acids in length is as follows (SEQ ID NO:1):

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T P L G P A R S L P Q S F L L K C L E Q V R K I Q A D G A E L Q E R L C A A H K

L C H P E E L M L L R H S L G I P Q A P L S S C S S Q S L Q L T S C L N Q L H G G

L F L Y Q G L L Q A L A G I S P E L A P T L D T L Q L D V T D F A T N I W L Q M

E D L G A A P A V Q P T Q G A M P T F T S A F Q R R A G G V L V A S Q L H R F

L E L A Y R G L R Y L A E P
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